SCIENTIFIC ABSTRACT

Although there is scientific and intuitive rationale for purging stem cell products to be infused as rescue following high-dose chemotherapy, methodologies are limited and not widely available. We propose to test a novel purging strategy in children with high-risk neuroblastoma, sarcomas, and retinoblastoma. This is a safety/phase II study measuring safety and time to engraftment following $CD34^+$ purification by two steps. We propose to determine the proportion of patients who achieve engraftment (defined as ANC $\geq 500/mm^3$ and platelets $\geq 10,000/mm^2$ without transfusion support) by day 56 in patients with high risk neuroblastoma or sarcoma following a sequential tumor purging strategy, that includes $CD34^+$ selection by immunomagnetic column followed by further $CD34^+$ purification by high speed cell sorting. Additionally, as secondary aims, we plan to evaluate the purging efficiency of this two-step selection by immunohistochemistry and/or quantitative RT-PCR for tumor specific transcripts, to evaluate purging efficiency by studying relapse specimens for the presence of the retroviral markers, to evaluate the efficiency of hematopoietic stem cell marking with retroviral vectors using two different marking procedures. Finally, we will evaluate the rate of engraftment and purging efficacy of this sequential purging procedure in pediatric patients with retinoblastoma.

PURGING STRATEGY

$CD34^+$ Antibody Selection

The CD34 antigen identifies an epitope characteristic of primitive hematopoietic progenitors that have the ability to reconstitute hematopoiesis. This allows the initial positive strategy of selecting for normal hematopoietic progenitors - a "reverse purge". Utilizing either the Baxter Isolex or Cellpro Ceprate systems, investigators have demonstrated a 1-3 log depletion of myeloma, breast, neuroblastoma, retinoblastoma and sarcoma cells. In peripheral blood mononuclear preparations "spiked" with neuroblastoma cells and $CD34^+$ selected, we demonstrated a similar purging efficacy utilizing fluorescent markers (sensitivity 1 tumor cell in 10^4 nucleated cells) and immunocytochemical detection (sensitivity 1 tumor cell in 10^5 nucleated cells) to quantitate residual neuroblastoma.

High Speed Cell Sorting

Many tumor cell types weakly express the CD34 antigen, prompting us to evaluate a further purging step. We performed pre-clinical studies to test and validate a sequential purging strategy utilizing a high-speed cell sorter. $CD34^+$ cells spiked with various tumor cell lines at a concentration of 1% were resorted for small $CD34^+$ cells by size exclusion criteria. This strategy resulted in a $CD34^+$ product that was 100% pure with a 50-60% yield. In addition, a 2-2.5 fold depletion of contaminating tumor cells was achieved . This was possible because the flow cytometric characteristics of tumor cells differ significantly from $CD34^+$ hematopoietic cells. This sequential purging accomplishes a 4-5 log depletion of tumor cells in vitro.